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Research Article

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Fibronectin in Human Bronchopulmonary Lavage Fluid

ELEVATION IN PATIENTS WITH INTERSTITIAL LUNG DISEASE

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ABSTRACT Fibronectin is a major adhesive and opsonic glycoprotein found in plasma and tissues. Because this molecule appears to mediate a number of interactions between cells and extracellular matrix, and because the interstitial lung diseases are characterized by marked derangements of the pulmonary extracellular matrix, we evaluated fibronectin in the lower respiratory tract in patients with these disorders. Fibronectin could be detected in the bronchoalveolar lavage fluid of normals (11/11), as well as those with noninterstitial lung diseases (18/18), idiopathic pulmonary fibrosis (21/21), sarcoidosis (20/20), and other interstitial lung diseases (22/22). Compared with normal and those with noninterstitial lung disease, the levels in bronchoalveolar lavage of patients with interstitial disease were significantly higher ($P < 0.01$, all comparisons). This was true only for bronchoalveolar lavage fibronectin; plasma levels were similar in all study groups ($P > 0.2$, all comparisons). The lavage fluid fibronectin was intact antigenically and retained collagen binding capability, although in some cases of interstitial disease, the presence of lower molecular weight fragments suggested some degradation. Thus, fibronectin is a normal constituent of the epithelial fluid of the lower respiratory tract and is present in increased amounts in a significant number of individuals with interstitial lung disease.

INTRODUCTION

Fibronectin is a large glycoprotein found in plasma and extracellular matrices (1–4). It is a major cell surface protein and is secreted by fibroblasts (5–6) and a variety of other cells (1, 7). Important properties of fibronectin include the fact that it binds to cell surfaces (8) and has other binding sites that recognize the collagen molecule (9) and other connective tissue components (10). In this context, fibronectin is thought to be involved in the interaction of cells with the extracellular matrix (11) and thus can influence both morphology (12–13) and differentiation (14–15). In addition, fibronectin has been implicated as an important opsonin for gelatin-coated particles and presumably for tissue debris in the circulation (16) and at local tissue sites (17).

The interstitial lung diseases are a heterogeneous group of disorders characterized by abnormal accumulation of inflammatory cells and connective tissue elements in the pulmonary interstitium together with changes in parenchymal cell populations and organization (18). Frequently these diseases lead to fibrosis characterized by derangements of interstitial collagen. Because fibronectin seems to play a major role in the maintenance of cellular and extracellular integrity and topography, it is reasonable to hypothesize that this macromolecule plays an important role in the derangements of the alveolar structures that characterize these disorders. In this context, the present study was designed to evaluate the status of fibronectin in the lower respiratory tract of patients with fibrotic lung disease. To accomplish this, fibronectin was measured in serum and bronchoalveolar lavage fluid of a group of patients with interstitial lung disease and compared with the values of normal individuals.

METHODS

Study population. 92 individuals were studied on the inpatient service at the Clinical Center, National Institutes of Health. Physiologic testing was performed as described (19).

21 (15 male, 6 female) had idiopathic pulmonary fibrosis (IPF)[1] in midcourse established by defined criteria including

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[1] *Abbreviations used in this paper:* D_{LCO}, diffusion capacity for carbon monoxide; ELISA, enzyme-linked immunoassay; FEV_1, forced expiratory volume at 1 s; FVC, forced vital capacity; IPF, idiopathic pulmonary fibrosis, TLC, total lung capacity; VC, vital capacity.

open lung biopsy (20). Five were smokers. 10 were taking prednisone (daily dose 27±6 mg)[2], and 4 were taking cytoxan (daily dose 125 mg). This group of patients had a mean age of 47.6±2.3 yr with a vital capacity (VC) of 60.0±4.6% predicted, total lung capacity (TLC) of 63.6±3.7% predicted, forced expiratory volume at 1 s (FEV_1) × 100/forced vital capacity (FVC) of 79.4±2.5% observed, single breath diffusion capacity for carbon monoxide (D_{LCO}) of 59.3±4.7% predicted (corrected for volume and hemoglobin), and resting arterial pO_2 of 72.5±2.9 torr. All IPF patients underwent bronchoalveolar lavage at least once. In addition, nine patients had two procedures and one patient had three; in these individuals, the average interval between procedures was 3 mo.

20 patients (9 male and 11 female) had pulmonary sarcoidosis diagnosed by established criteria including lung biopsy (21). One was a smoker. Four were taking prednisone (daily dose 31±16 mg), and four were receiving 2 gm i.v. solumedrol weekly. These patients had a mean age of 40.1±3.0 yr; VC of 81.8±3.0% predicted, TLC of 81.0±3.1% predicted, FEV_1/FVC% of 75.3±2.3% observed, D_{LCO} of 95.3±3.1% predicted, and resting PaO_2 of 87.0±3.0 torr. These 20 sarcoidosis patients underwent 28 lavage procedures. 16 patients had one, 2 had two, 1 had three, and 1 patient had five procedures. The average interval between multiple procedures was 2 mo.

22 patients (11 male and 11 female) were classified as having other interstitial lung diseases using established criteria (18). These included patients with eosinophilic granuloma (n = 4), hypersensitivity pneumonitis (n = 4), chronic interstitial lung disease associated with collagen vascular disease (n = 5), Wegener's granulomatosis (n = 2), pulmonary alveolar proteinosis (n = 2), asbestosis (n = 2), chronic eosinophilic pneumonia (n = 1), and undiagnosed chronic interstitial lung disease (n = 2). Six were smokers. Four were taking prednisone (daily dose 30±11 mg). One was taking cytoxan (50 mg/d). This group had a mean age of 45.4±3.1 yr with VC of 70.2±4.2% predicted, TLC of 75.1±3.4% predicted, FEV_1/VC% of 75.3±2.7% observed, D_{LCO} of 75.1±5.0% predicted, and resting PaO_2 of 82.8±3.0 torr. Of this group, 18 patients had one, 2 patients had two, and 1 patient had three lavage procedures. The average interval between multiple procedures was 2.5 mo.

For comparison with the patients with interstitial disease, two groups of control patients were evaluated. The first group consisted of 11 (4 male, 7 female) normal volunteers. Three were smokers. Mean age for this group was 27.6±4.6 yr, VC of 90.5±2.7% predicted, TLC of 86.7±2.8% predicted, FEV_1/FVC was 86.2±2.5% observed, D_{LCO} of 104.5±4.7% predicted, and PaO_2 of 92.2±4.3 torr. The second control group consisted of 18 patients (13 male, 5 female) who had a variety of noninterstitial lung disorders including: chronic obstructive airways disease (n = 10), localized metastatic disease (n = 3), localized infiltrates likely due to viral infection (n = 2), and hemoptysis of unknown etiology (n = 3). Seven were smokers. Four were taking prednisone (daily dose 52±18 mg/d). The group had a mean age of 45.6±3.7 yr, VC of 83.1±5.3% predicted, TLC of 95.4±4.5% predicted, FEV_1/FVC of 81.2±4.8% observed, D_{LCO} of 85.7±6.4% predicted, and PaO_2 of 85.7±4.8 torr. Each control individual had one procedure.

Bronchoalveolar lavage. Bronchoalveolar lavage was performed with a flexible fiberoptic bronchoscope as described (22) using a total of 100 ml of 0.9% saline in five 20-ml aliquots for each site lavaged. No patient was lavaged who had clinical evidence of acute pulmonary infection. The samples were centrifuged at 2,000 g for 15 min to remove cellular material and debris. Four 1-ml aliquots of the supernatant fluid were then frozen on dry ice and stored in liquid nitrogen vapor. Each aliquot was used only once. Plasma samples, anticoagulated with ethylenediaminetetraacetic acid, were collected the morning of bronchoscopy. Plasma samples were centrifuged at 2,000 rpm for 15 min to remove cells, and three 1-ml aliquots were frozen on dry ice and stored in liquid nitrogen vapor until use.

Quantification of fibronectin levels in lavage fluid and plasma. All measurements were done by enzyme-linked immunoassay (ELISA) as described (23). Briefly, fibronectin purified from human plasma by affinity chromatography and diethylaminoethyl-cellulose chromatography was used to raise antifibronectin antisera in rabbits. The second antibody was peroxidase-conjugated goat antirabbit immunoglobulin (IgG) (lot S-631) obtained from Miles Laboratories Inc., Elkhart, Ind. The same ELISA assay was used to quantify lavage fluid and plasma fibronectin; to standardize the assay[2] plasma and lavage fluid fibronectins were purified as described above and compared using ELISA. The concentration of protein in the purified fibronectin sample was determined using a protein-binding dye assay (Bio-Rad Laboratories, Richmond, Calif.); the ELISA test was sensitive to 10 ng/ml.

To compare fibronectin levels in lavage among different study groups, fibronectin levels in lavage are given relative to lavage fluid albumin levels. This is necessary to correct for the variable amount of dilution of the lower respiratory tract sample by the saline used in the lavage. Because albumin (67,000 mol wt) is representative of an average-sized molecule in terms of movement across the alveolar-capillary barrier, this provides a convenient measure to compare lavage fluid macromolecule levels among groups and to compare lavage fluid with plasma levels (24, 25). This internal albumin control is necessary to correct for the variable amount of dilution that occurs during the lavage procedure. Albumin was measured using a similar ELISA test. Crystalline human serum albumin (lot 36), goat antihuman albumin (lot G-389), and peroxidase-conjugated rabbit antigoat IgG (lot S-605) were obtained from Miles Laboratories Inc. For this assay, 400 ng albumin was used to coat each well of the micro ELISA plate (Dynatech Laboratories, Inc., Alexandria, Va.). Inhibition tests were done with goat antihuman albumin at a dilution of 1:2,500. The test was sensitive to 30 ng/ml. All fibronectin/albumin data were expressed as micrograms per milligram. To further validate the use of the fibronectin/albumin ratio in several patients, the standard 100-cm^3 lavage was collected in five separate 20-cm^3 aliquots, which were processed separately.

Lobar distribution of fibronectin. To compare the lobar distribution of fibronectin in the same individual, lavages of multiple lobes during the same bronchoscopy procedure were made in 70 individuals in the study population. 38 lavages were performed in the lingula, left lower lobe, and right middle lobe. In 32 individuals, lavages were made in two separate lobes. All samples were processed separately.

Evaluation of form and function of lavage fluid fibronectin. To determine whether lavage and plasma fibronectin were antigenically similar or different, a standard curve for the ELISA was generated using lavage fluid, purified lavage fibronectin, and plasma fibronectin. The standard curves from the ELISA were calculated by the method of Rodbard (26) on a Honeywell 1170 computer (Honeywell, Inc., Denver, Colo.).

[2] All data is presented as mean±SE of the mean.

To evaluate the function of lavage fluid fibronectin two methods were used.

(*a*) To compare the collagen binding of antigenic lavage fluid fibronectin to collagen to that of plasma fibronectin, a modification of the method of Engvall and Ruoslahti (27) was used. Microtiter wells were coated with type I collagen (200 ng/well) in Voller's buffer as described above. After rinsing, 200-μl samples of lavage fluid and plasma were added to each well and incubated for 30 min at 23°C. The unbound material was rinsed away and rabbit antifibronectin antibody (diluted 1:1,000) was added to each well. This was then incubated for 90 min at 23°C to allow antibody to bind to any antigenic material that had bound to the collagen-coated well. Next, horse radish peroxidase-conjugated goat antirabbit IgG was added, followed by peroxidase substrate, and the resulting chromaphore produced was measured as described above.

(*b*) Quantitative binding of lavage fluid fibronectin to gelatin-Sepharose was done batchwise. The gelatin-Sepharose was prepared as described by Engvall and Ruoslahti (27). Affinity beads suspended in phosphate-buffered saline (PBS) (1:1 beads to PBS; 0.5 ml total) was added to 0.75 ml of sample (lavage or appropriately diluted plasma) in Eppendorf tubes. Samples were gently mixed for 1 h at room temperature and were then centrifuged at 10,000 *g* for 10 min. Under these conditions, the percentage of fibronectin bound in a given sample was constant over a range of 50 to 1,000 ng/ml (data not shown). Aliquots of the supernatant fluid and starting material were then assayed for fibronectin by ELISA under conditions that permit the detection of intact and partially cleaved molecules (28). The percentage of fibronectin bound was calculated as [fibronectin in the starting sample − fibronectin in the supernatant × (1.67)] × [100]/[fibronectin in the starting sample]. The factor of 1.67 is used to correct the dilution of the fibronectin in the supernatant by the gelatin-Sepharose beads in PBS.

To evaluate the apparent molecular weight of the lavage fluid fibronectin that could bind to gelatin, lavage samples were iodinated using lactoperoxidase-glucose oxidase beads (Bio-Rad Laboratories) and $Na^{125}I$ (New England Nuclear, Boston, Mass.). 50-μl aliquots of each iodinated sample was purified using 50 μl of gelatin-Sepharose beads as described above. The centrifuged beads were suspended in 100 μl of sample buffer with 2% β-mercaptoethanol and heated to 100°C for 5 min. For comparison, aliquots of each lavage fluid sample were precipitated with 1 ml of 20% trichloroacetic acid followed by a wash with 1 ml of 99% ethanol at −20°C. Precipitates, collected by centrifugation at 10,000 *g* for 10 min, were dissolved in gel buffer and electrophoresed in sodium-dodecyl sulfate (SDS)-polyacrylamide slab gels (5%) (29). After electrophoresis, the gels were stained, destained, and autoradiograms were made.

Statistical evaluation. All comparisons between groups were made using the Wilcoxon-Mann Whitney rank order test except for the evaluation of the lobar distribution of fibronectin, which was evaluated in 2 × 2 tables using the chi squared test.

RESULTS

Fibronectin levels in bronchoalveolar lavage fluid. To demonstrate that the ELISA accurately quantified lavage and plasma fibronectin in comparable fashion, the ELISA was used to evaluate fibronectin levels in increasing amounts of lavage fluid and compared with both fibronectin purified from the patient's lavage fluid and to plasma fibronectin (Fig. 1). Not only was fibronectin detected in lavage fluid, but the shape of the lavage and plasma ELISA curves were identical, demonstrating that lavage and plasma fibronectin are antigenically indistinguishable in the ELISA. Moreover, aliquots of lavage fluid fibronectin purified by gelatin-Sepharose binding also inhibited the ELISA in

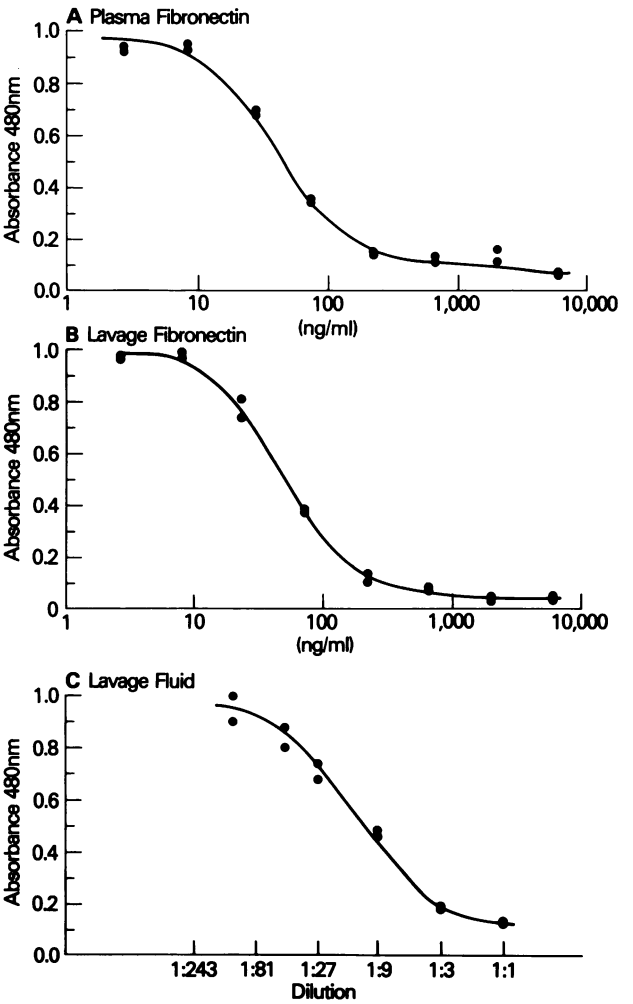

FIGURE 1 Antigenic identity of lavage fluid fibronectin and plasma fibronectin in the ELISA test. Increasing quantities of (A) plasma fibronectin, (B) lavage fluid fibronectin, and (C) unpurified lavage fluid were evaluated using an indirect ELISA assay (see Methods for details). The identity of the shape of the ELISA curves suggests they are antigenically similar. The curves were fit by a computer program described by Robard (26). Ordinate in all panels: measured absorbance at 480 nm of enzyme product in the ELISA. Abscissa: Panel A: standard plasma fibronectin concentration in nanograms per milliliter; Panel B: purified lavage fluid fibronectin in nanograms per milliliter; Panel C: total lavage fluid in arbitrary dilutional units.

a parallel fashion (Fig. 1). Although this does not exclude minor chemical differences not reflected in the antigenic nature of the fibronectin as detected in the ELISA, the test can be used to measure both lavage fluid and plasma fibronectin. Moreover, the ratio of fibronectin/albumin during sequential lavages of the same bronchopulmonary segment was constant (Fig. 2). This was true even though the recovery of the proteins varied during the procedure. Thus, this ratio provides a valid quantitative measure of lower respiratory tract fibronectin.

Fibronectin was evaluated in 260 lavage samples during 148 bronchoalveolar lavage procedures of 92 study individuals. Using the ELISA assay with a detection limit of 10 ng/ml, fibronectin was detected in the lavage fluid of all study individuals (Fig. 3). In normals, lavage fluid fibronectin was 1.6±0.4 μg/mg albumin (range 0.3 to 3.1 μg/mg albumin). In patients with noninterstitial lung diseases, the lavage fluid fibronectin was 2.1±0.3 μg/mg albumin ($P > 0.2$ compared with normal). In contrast, the mean level of fibronectin in IPF, sarcoidosis, and other interstitial lung disorders was significantly higher than in controls (IPF 4.3±0.9, range 0.3 to 13.6 μg/mg albumin, $P < 0.001$ compared with controls; sarcoidosis 5.4±1.0, range 0.3 to 20.2 μg/mg albumin, $P < 0.01$ compared with controls; other interstitial lung diseases 4.3±0.8, range 0.4 to 13.5 μg/mg albumin, $P < 0.01$ compared with controls).

Lavage fluid levels did not correlate with smoking history. For the population as a whole, nonsmokers ($n = 69$) had a mean fibronectin level of 3.7±0.4 μg/mg albumin, and smokers ($n = 21$) had a mean fibronectin level of 3.5±0.7 μg/mg albumin, $P > 0.2$. There was also no association with smoking in any patient group within the population, but the numbers of smokers in most groups were too small for definitive statistical analysis. Similarly, there was no correlation with prednisone therapy. (The fibronectin level for patients not taking prednisone was 4.25±.58 μg/mg albumin vs. a level of 4.07±0.98 μg/mg albumin for patients taking prednisone, $P > 0.2$).

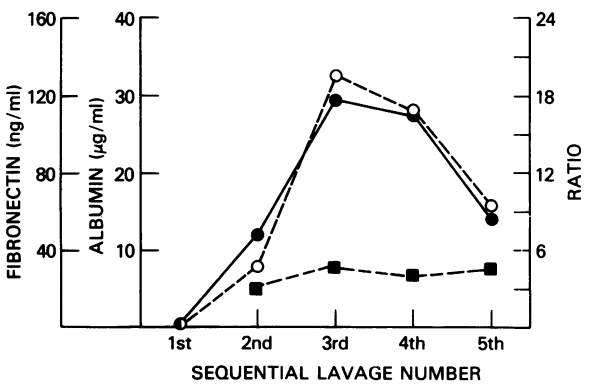

FIGURE 2 Constancy of fibronectin/albumin ratio in bronchoalveolar lavage in the face of variable recovery. Sequential 20-ml lavages were performed in the same bronchopulmonary segment, and all samples were handled separately. Ordinate: fibronectin concentration in nanograms per milliliters, (O —— O); albumin concentration in μg/ml, (● —— ●); ratio of fibronectin to albumin (units = nanograms per milliliter per micrograms per milliliter, ■ —— ■). Abscissa: lavage sequence number.

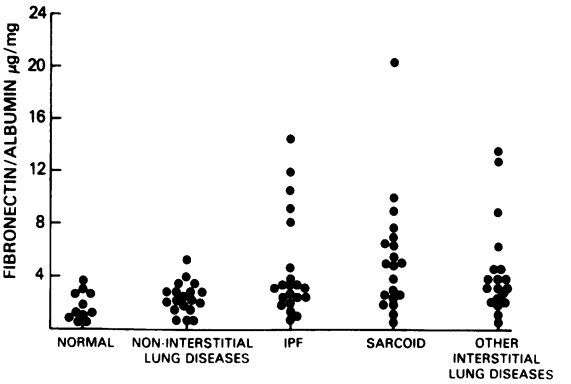

FIGURE 3 Fibronectin levels in bronchoalveolar lavage fluid of patients with interstitial lung disease. Each data point represents lavage of a single patient; when multiple lavages were performed, the mean value was used. All data is presented as fibronectin (micrograms)/albumin (milligrams) in lavage fluid. Other lung disease = patients with noninterstitial lung disease. IPF = patients with idiopathic pulmonary fibrosis; sarcoid = patients with pulmonary sarcoidosis; and ILD = patients with a variety of other interstitial lung disorders.

The elevation of lavage fluid fibronectin in interstitial lung disease was in marked contrast to the plasma levels of fibronectin in the same individuals. For all groups, plasma fibronectin was within the normal range independent of whether the values were expressed as micrograms fibronectin per milliliter plasma (Fig. 4A) or micrograms fibronectin per milligram plasma albumin (Fig. 4B) ($P > 0.2$, all comparisons). Comparison of lavage with plasma levels of fibronectin revealed that the lavage fluid level was 25±3% of plasma in normals and 23±7% in patient controls with noninterstitial lung diseases ($P > 0.2$). In contrast, it was 44±7% in patients with IPF, 76±12% in sarcoidosis, and 71±18% in patients with other interstitial lung diseases ($P < 0.01$ for all comparisons to control). Moreover, four patients with IPF, four patients with sarcoidosis, and two patients with other interstitial lung diseases had lavage levels that exceeded 100% of the plasma level suggesting that, in at least some individuals with interstitial disease, fibronectin in lavage is either produced or concentrated locally, or removed more slowly.

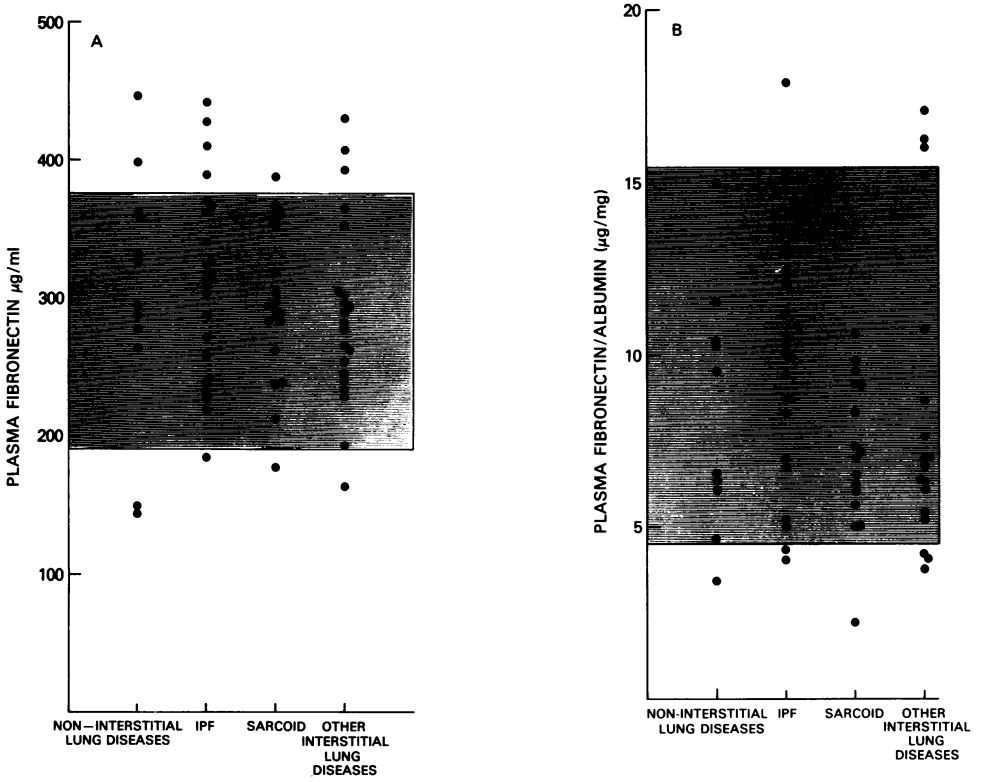

FIGURE 4 Plasma fibronectin levels in patients with interstitial lung disease. (A) Data presented as fibronectin (micrograms) per plasma volume (milliliters). (B) Data presented as fibronectin (micrograms) per plasma albumin (milligrams). In both panels, the normal range in our laboratory (mean±2 SD) is indicated by the crosshatched region. Other lung disease = patients with noninterstitial lung disease. IPF = patients with idiopathic pulmonary fibrosis; sarcoid = patients with pulmonary sarcoidosis; and ILD = patients with a variety of other interstitial lung disorders.

Lobar distribution of fibronectin. To determine if the elevated fibronectin levels in bronchoalveolar lavage of patients with interstitial disease was a generalized phenomenon throughout the lung, 70 patients were lavaged in multiple subsegments (Table 1). 38 had separate lavages in the lingula, left lower lobe,

TABLE I
Concordance of Lobar Distribution of Fibronectin•

A	Lingula‡		B	Lingula§		C	Left lower lobe‖	
Left lower lobe	Low values	High values	Right middle lobe	Low values	High values	Right middle lobe	Low values	High values
Low values	28	5	Low values	32	6	Low values	25	3
High values	6	12	High values	6	9	High values	4	9

• Comparison of the ratio of the fibronectin to albumin (micrograms per milligram) of patients lavaged in multiple sites during the same procedure. The numbers in each 2 × 2 comparison refer to the number of individuals with low values of fibronectin/albumin (<3.5 μg/mg) or high values of fibronectin/albumin (≥3.5 μg/mg) in lavage fluid of each lobe.

‡ Concordance (compared with random distribution) of lingula vs. left lower lobe, $P < 0.003$; left lower lobe vs. right middle lobe, $P < 0.0025$.

§ Concordance (compared with random distribution) of lingula vs. right middle lobe, $P < 0.0018$.

‖ Concordance (compared with random distribution) of left lower lobe vs. right middle lobe, $P < 0.0025$.

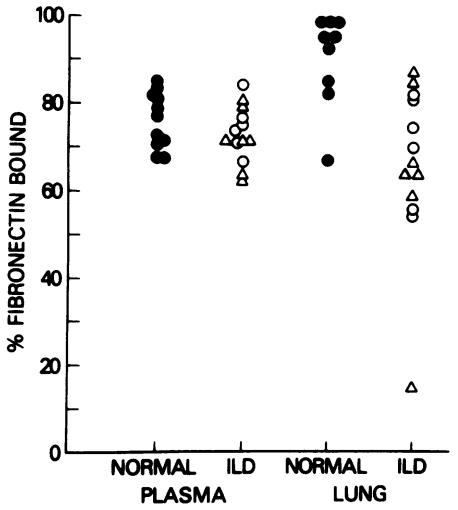

FIGURE 5 Quantitative evaluation of the ability of lavage fluid fibronectin to bind to denatured collagen. Lavage fluid was added to gelatin-coated Sepharose beads; after incubation for 1 h at 23° the amount of fibronectin remaining in the supernatant was quantitated by ELISA. Data is presented as percent fibronectin in starting material that bound to the gelatin-Sepharose. Shown are normals (●) and patients with interstitial lung disease (ILD); the latter included individuals with idiopathic pulmonary fibrosis (○) and sarcoidosis (△). Data for plasma is shown for comparison.

and right middle lobe during the same procedure. In 32 patients, adequate lavage samples were obtained in only two lobes. All lavages were scored as having elevated fibronectin/albumin ratio if the value was ≥ 3.5, i.e., a level that was greater than all normal values. Comparisons were then made for concordance among the three lobes. For all possible combinations, there was a statistically significant concordance among the lobes, suggesting uniformity in fibronectin levels throughout the lung.

Evaluation of structure and function of bronchoalveolar lavage fluid fibronectin. Evaluation of the function of lavage fluid fibronectin demonstrated that a variable amount was capable of binding to denatured collagen (Fig. 5). Whereas from 68 to 86% of plasma fibronectin from controls was capable of binding to gelatin-coated Sepharose, 68–100% of lavage fluid from the same individuals could perform the same function. In contrast, while at least 68% of lavage fluid fibronectin from normals was capable of binding to denatured collagen, in 46% (6 of 13) patients with interstitial lung disease, <68% of fibronectin detected antigenically in lavage fluid maintained this property.

The lavage fluid fibronectin that bound to collagen reacted with the antibody in a manner similar to plasma fibronectin from the same patients suggesting a similar antigenic character (Fig. 6). At least some of the lavage fluid fibronectin that bound to collagen was intact; evaluation of iodinated lavage fluid fibronectin by SDS-acrylamide gels demonstrated an apparent monomer mol wt of 220,000 (Fig. 7). For one IPF patient (lane 2), a molecule of approximate mol wt of 80,000 adsorbed to the gelatin-Sepharose beads (band

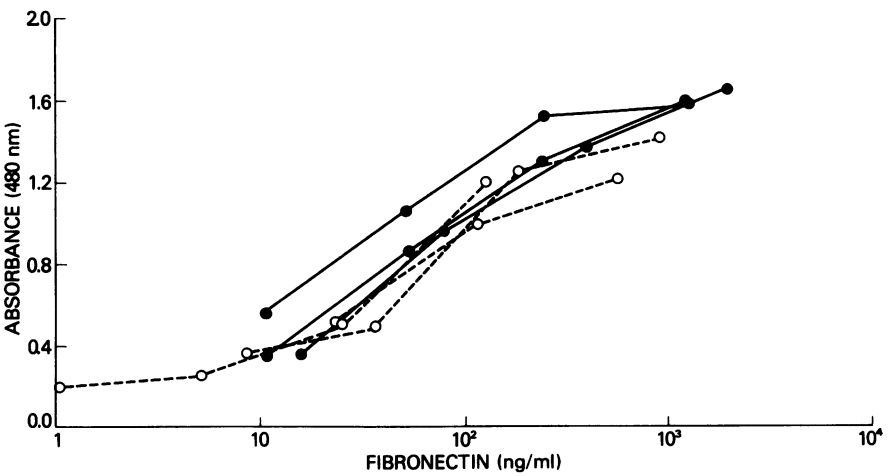

FIGURE 6 Antigenic activity of lavage fluid bound to collagen. Microtiter wells were coated with collagen as described in Methods. Various amounts of lavage fluid and plasma fibronectin were then added and allowed to bind, following which the antigenic activity was determined by adding rabbit antifibronectin antibody as described in Methods. The antigenic activity of bound material was then determined by adding peroxidase-conjugated goat antirabbit IgG and quantifying enzyme product. Shown are plasma (● —— ●) and lavage (○ —— ○) samples. Ordinate: absorbance of enzyme product generated; Abscissa: amount of total fibronectin in sample.

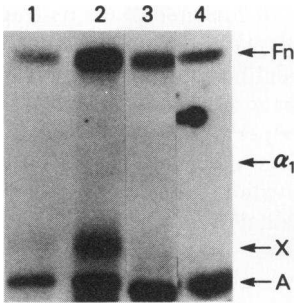

FIGURE 7 Evaluation of the intact fibronectin in lavage fluid by SDS-polyacrylamide gels. Lavage fluid from three patients with interstitial lung disease (lane 1: asbestosis, lane 2: idiopathic pulmonary fibrosis, lane 3: sarcoidosis) was labeled with ^{125}I and exposed to gelatin-Sepharose. The bound material was eluted in the gel-sample buffer and electrophoresed as described in Methods. For comparison, plasma from patient 2 (lavage fluid in lane 2) underwent the same treatment and is shown in lane 4. Lavages and plasma from control individuals handled in a similar manner also demonstrated intact fibronectin (data not shown). Molecular weight markers fibronectin ("fn"), collagen α1 chains ("α1"), and albumin ("A") are shown. The band at A is likely ^{125}I-albumin adsorbed to or trapped by the gelatin-Sepharose beads and "X" = likely a collagen-binding fragment of fibronectin (patient in lane 2). The assignment of A as albumin was made because: (1) it has the correct molecular weight; and (2) this was the major protein (>70% of all dpm) in trichloroacetic acid precipitates of unfractionated iodinated lavage fluid, consistent with the known fact that albumin represents ~30% with surfactant proteins (not shown) comprising ~30% of proteins recovered by lavage. The assignment of X as a fibronectin fragment was made because it was not seen in unfractionated iodinated lavage fluid analyzed by SDS-gels (not shown) but was markedly enriched by affinity binding to gelatin-Sepharose, a known property of intact fibronectin and some of its fragments.

X) and might represent a fragment of fibronectin with an intact collagen binding site. Thus, at least some of the lavage fluid fibronectin of both controls and patients with interstitial lung disease is similar antigenically to plasma fibronectin and has the same apparent monomer molecular weight as plasma fibronectin. Moreover, this material binds to collagen and thus possesses at least one of the functional characteristics of plasma fibronectin. With the material available, it was not possible to evaluate the cell binding function of lavage fluid fibronectin because the standard assay for cell binding required fibronectin levels in excess of 1 μg/ml, whereas lavage fluid contained fibronectin 0.1–1 μg/ml in most individuals. Preliminary attempts to concentrate lavage fluid to permit cell binding evaluation were unsuccessful. Although the reasons for this are unclear, it likely was secondary to the lavage fluid fibronectin precipitating or aggregating under the concentrating conditions.

DISCUSSION

Fibronectin is a large glycoprotein found in plasma, interstitial connective tissue, basement membranes, and cell surfaces (1–7). Through its ability to bind to connective tissue and cells (9), this macromolecule mediates many cell-matrix interactions (1, 4, 11), including cell adhesion (30), the clearance of denatured matrix components (4, 16, 17), and the directed movement of parenchymal cells (31). In this context, fibronectin is likely important in situations where cellular and matrix constituents are being remodeled. Using the technique of bronchoalveolar lavage (24) to sample fibronectin in the lower respiratory tract, our study demonstrates that fibronectin is normally present in the lower respiratory tract, and significantly increased levels of this macromolecule can be recovered from the lungs of patients with interstitial lung disease, disorders characterized by chronic remodeling of alveolar structures.

Fibronectin in human bronchoalveolar lavage fluid. Fibronectin can be easily detected in the fluid lining the epithelial surface of the human lower respiratory tract. This is not surprising, as fibronectin has been described in other body fluids, including cerebral spinal fluid (32) and amniotic fluid (33). In addition, Bray et al. (34) have isolated fibronectin directly from the lung parenchyma and several investigators have demonstrated fibronectin in the alveolar structures by immunofluorescence methods (2, 3).

There are several possible mechanisms to explain the presence of fibronectin on the epithelial surface of the lower respiratory tract of the human lung. First, it may represent, in part, fibronectin that has permeated from plasma across the alveolar-capillary structures. In normal individuals, the level of fibronectin/albumin in lavage was an average of 25±3% of that of plasma. Although lung permeability studies with fibronectin have not been carried out, plasma fibronectin has a mol wt of 440,000, and it is known that macromolecules of that size can diffuse from blood into lung, although not as readily as albumin (35, 36). Second, fibronectin is a major secretory product of lung fibroblasts (1, 5, 6), a cell population that represents 35–40% of the parenchymal cells of the normal lung (37). Third, fibronectin is produced by alveolar macrophages (38, 39), a cell representing 90% of the inflammatory and immune effector cells of the lower respiratory tract.

Independent of the source of the fibronectin recovered by lavage of the human lung, at least some of this material is similar to other fibronectins. For example, it is antigenically indistinguishable from plasma fibronectin. In addition, at least some of it has a monomer mol wt of 220,000 and binds to collagen,

both of which are properties of other fibronectins (1, 9, 27). There have been subtle differences described between cellular fibronectin and plasma fibronectin (1, 40), but because the amounts of human lavage fluid fibronectin available are very limited, extensive biochemical and functional comparison to plasma fibronectin is not possible at this time.

Lavage fluid fibronectin in interstitial lung disease. More than half of the individuals with intersitial lung disease have lavage fluid fibronectin levels that are greater than the highest level observed in lavage fluid in normals (Fig. 3). Interestingly, the same individuals have plasma fibronectin levels similar to normals (Fig. 4), suggesting the increases seen in lung represent a local process: either production or concentration of fibronectin at the site of disease activity. In contrast, only 2 of 18 patients with noninterstitial lung disease, had fibronectin levels higher than the highest normal, and there was no statistical difference between this patient group and normals. Thus the elevation of fibronectin level in interstitial disease appears to reflect the presence of a characteristic group of disease processes of the alveolar structures. Moreover, the elevated lavage fluid fibronectin found in interstitial disease appears to be a phenomenon generalized throughout the lung, i.e., fibronectin levels in lavage fluid from various lobes of the same patient are similar (Table I). This does not imply that the elevation is completely uniform, but rather that the lavage procedure samples a sufficiently large number of alveoli so that in a generalized process segmental levels are similar.

Whereas fibronectin appears to be a ubiquitous protein produced by many cell types and present throughout the body, it is very unusual to find abnormalities in the levels of fibronectin in body fluids. Besides the elevated levels of lavage fluid fibronectin in interstitial disease noted in this study, the only other variations in fibronectin levels noted in human disease are the reduction in plasma fibronectin associated with massive trauma (4) and disseminated intravascular coagulation (1, 41), possibly a mild elevation in plasma levels of some patients with hepatic dysfunction (42), and possibly abnormal levels in the cerebrospinal fluid of some patients with neurologic disorders (32).

Although some of the increased levels of fibronectin in the lower respiratory tract of patients with interstitial disease may result from an increased "leak" of plasma macromolecules due to the derangement of the alveolar-capillary structures, lavage fluid levels of other high molecular weight macromolecules found in blood are not elevated in these disorders (22, 25). Moreover, 21% of individuals with interstitial lung disease had a lavage to plasma fibronectin/albumin ratio of >1.0, a situation that clearly implies either local generation of fibronectin (or its fragments), local concentration of plasma fibronectin, or decreased removal of fibronectin from the lower respiratory tract. In addition, because there is little evidence of increased selective permeability of the capillary-alveolar membrane to large macromolecules in interstitial lung disease (22), elevated levels of lavage fluid fibronectin in many individuals likely reflects local processes at the site of disease.

There are several possible mechanisms to explain increased local generation of fibronectin in the lower respiratory tract of patients with interstitial lung disease. First, fibronectin is a major secretory product of fibroblasts (1, 5, 6), a cell population that is expanded in the parenchyma of the lungs of these patients (17). Second, at least some of the interstitial lung diseases are associated with the presence of proteolytic enzymes in the lower respiratory tract (43), thus providing a means by which tissue fibronectin or its fragments could be released to the epithelial surface (35, 44, 45). Consistent with this concept, some of the antigenic fibronectin found in the lavage fluid of patients with interstitial lung disease is nonfunctional (i.e., binding to collagen is reduced) (Fig. 5). In addition, some of the fibronectin in lavage bound to collagen but was of lower molecular weight (i.e., likely the collagen-binding site was intact but not the entire molecule) (Figs. 6, 7), and evaluation of lavage fluid of interstitial lung disease patients by molecular sieve analysis demonstrated significant amounts of antigen of smaller molecular weight than the fibronectin monomer (data not shown). Increased degradation of fibronectin in the lung in the interstitial diseases is consistent with the accelerated turnover of other connective tissue macromolecules in these diseases and, if at times degradation exceeds production, may explain some of the overlap of patient samples with controls. Third, macrophages (46, 47), including alveolar macrophages (39, 40), are capable of producing fibronectin. This cell population is expanded in number in the interstitial lung diseases (17), and macrophages from these patients produce increased amounts of fibronectin per cell (40).

No association between fibronectin level and pulmonary function was observed. However, pulmonary function testing does not measure the activity of the interstitial disease but merely its end result (18, 48–50). Fibronectin itself would not be expected to alter pulmonary function, but rather, to reflect disease process. Moreover, in this context, the metabolism of fibronectin is the important parameter, and the level measured in bronchoalveolar lavage must represent a balance between production and destruction. These dynamic processes and the possible predictive value of the fibronectin level for morphological and func-

tional outcome in these diseases will have to await long-term followup studies in individual patients.

The increased levels of fibronectin on the epithelial surface of the lower respiratory tract of patients with interstitial lung disease may simply be a byproduct of the active inflammation associated with these disorders and not relate to their pathogenesis. However, the increased amounts of fibronectin in the alveolar structures may play an important role in the chronic remodeling of the lung parenchyma that is characteristic of these diseases. First, by virtue of its function as an opsonin (4), fibronectin might provide a mechanism whereby the lung removes matrix debris after injury. Second, fibronectin is known to be a chemoattractant for fibroblasts (31) and could thus recruit these cells to local areas of injury in the alveolar structures. Moreover, by functioning as an adhesive protein (1, 8, 10, 29), fibronectin can influence the cellular topography of the injured tissue. Third, fibronectin has been implicated as a possible modulator of phenotypic expression for certain cell types (13, 14). In this context, it is possible that the fibronectin in the lower respiratory tract of patients with interstitial lung disease may influence, in part, the function of the parenchymal cells present.

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